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Cephalezomines G, H, J, K, L, and M, new alkaloids from *Cephalotaxus harringtonia* var. *nana*

Hiroshi Morita, Miwa Yoshinaga and Jun'ichi Kobayashi*

Graduate School of Pharmaceutical Sciences, Hokkaido University, Sapporo 060-0812, Japan

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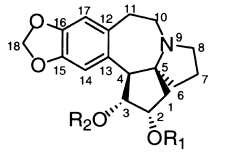
Abstract—Five new cephalotaxine-type alkaloids, cephalezomines G, H, J, K, and L (**1**–**5**), and a new homoerythrina-type alkaloid, cephalezomine M (**6**), have been isolated from the leaves of *Cephalotaxus harringtonia* var. *nana*. The relative and absolute stereochemistry was elucidated by NOESY data, CD analysis applying exciton chirality methods, and chemical means.

Cephalotaxus alkaloids are a family of cytotoxic heterocyclic natural products elaborated by trees of the genus *Cephalotaxus* (Cephalotaxaceae), some of which showed antileukemic activity.[1] Recently, we have isolated six new cytotoxic alkaloids, cephalezomines A–F,[2] from the leaves of *Cephalotaxus harringtonia* var. *nana* and a novel pentacyclic alkaloid, cephalocyclidin A,[3] from the fruits of the same plant. Our continuing search for structurally unique and biogenetically interesting *Cephalotaxus* alkaloids resulted in the isolation of cephalezomines G, H, J, K, and L (**1**–**5**), five new cephalotaxine-type alkaloids, and cephalezomine M (**6**), a new homoerythrina-type alkaloid, from the leaves of *C. harringtonia* var. *nana*. Here, we describe the isolation and structure elucidation of **1**–**6**.



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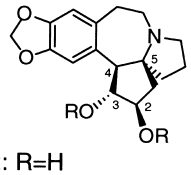
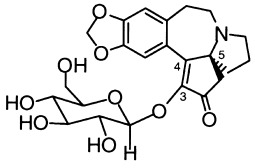
7: R_1=binaphtyl, R_2=H
8: R_1=H, R_2=binaphtyl



2: R=H
9: R=*p*-methoxycinnamoyl



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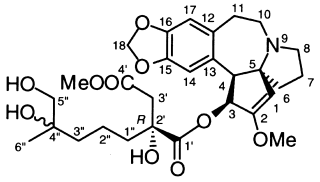
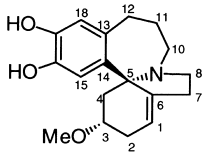


4 and **5** (epimers at C-4")



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The leaves of *C. harringtonia* var. *nana* were extracted with MeOH, and the MeOH extract was partitioned between AcOEt and 3% tartaric acid. Water-soluble materials adjusted at pH 10 with sat. Na_2CO_3 aq. were partitioned with $CHCl_3$. $CHCl_3$-soluble materials were subjected to a silica gel column ($CHCl_3$/MeOH, 1:0→0:1, and then *n*-BuOH/AcOH/H_2O, 4:1:1), in which a fraction eluted with BuOH/AcOH/H_2O (4:1:1) was purified by C_{18} HPLC (15% CH_3CN/0.1%TFA) to afford cephalezomines G (**1**, 0.00004% yield) and H (**2**, 0.00002%). In the silica gel column, a fraction eluted with MeOH was purified by C_{18} HPLC (20% CH_3CN/0.1%TFA) to afford cephalezomines J (**3**, 0.00002%), K (**4**, 0.00003%), L (**5**, 0.00004%), and M (**6**, 0.00004%).

Cephalezomine G {**1**, $[\alpha]_D$=−48° (*c* 1.8, MeOH)} was revealed to have the molecular formula, $C_{17}H_{21}NO_4$, by HRFABMS [*m*/*z* 304.1539 $(M+H)^+$, Δ−1.0 mmu]. The IR absorption implied the presence of hydroxyl (3395 cm^{-1}) group(s). Analysis of ^1H and ^{13}C NMR data (Tables 1 and 2, respectively) and the HMQC spectrum provided evidence that **1** possessed 17 carbon signals including 5 quaternary carbons (sp^2×4 and sp^3×1), 5 methines (sp^2×2 and sp^3×3), and 7 methylenes. Among them, two methines (δ_C 77.89 and 82.83) and one methylene (δ_C 102.68) were ascribed to

Keywords: cephalotaxine; homoerythrina; alkaloids; *Cephalotaxus harringtonia* var. *nana*.

* Corresponding author. Tel.: +81-11-706-3239; fax: +81-11-706-4985; e-mail: jkobay@pharm.hokudai.ac.jp

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Table 1. ^{1}H NMR data [δ_H (J, Hz)] of cephalezomines G (**1**), H (**2**), J (**3**), and M (**6**) in CD_3OD at 300 K

	1	2	3	6
1	1.93 (2H, m)	2.01 (1H, m) 2.39 (1H, m)	3.07 (2H, s)	6.05 (1H, s)
2	4.29 (1H, d, 4.2)	4.23 (1H, m)		2.08 (1H, m) 2.72 (1H, m)
3	4.23 (1H, d, 6.6)	4.16 (1H, dd, 5.6, 4.9)		3.28 (1H, m)
4	3.69 (1H, d, 6.6)	3.48 (1H, d, 5.6)		1.77 (1H, t, 11.4) 2.84 (1H, m)
5				
6	1.93 (1H, m) 2.81 (1H, m)	1.92 (1H, m) 2.39 (1H, m)	2.28 (2H, m)	
7	1.93 (1H, m) 2.10 (1H, m)	1.92 (1H, m) 2.11 (1H, m)	2.41 (2H, m)	2.72 (2H, m)
8	3.19 (1H, m) 3.52 (1H, m)	3.20 (1H, m) 3.54 (1H, m)	3.57 (1H, m) 4.01 (1H, m)	2.72 (1H, m) 3.35 (1H, s)
10	3.31 (2H, m)	3.35 (1H, m) 3.41 (1H, m)	3.52 (1H, m) 3.83 (1H, m)	3.51 (1H, d, 14.2) 3.77 (1H, m)
11	2.52 (1H, m) 4.21 (1H, m)	2.58 (1H, m) 4.31 (1H, m)	3.30 (2H, t, 3.8)	1.94 (1H, m) 2.08 (1H, m)
12				3.17 (1H, t, 13.0) 3.28 (1H, m)
13				
14	6.82 (1H, s)	6.81 (1H, s)	7.26 (1H, s)	
15				6.75 (1H, s)
16				
17	6.80 (1H, s)	6.80 (1H, s)	6.98 (1H, s)	
18	5.94 (2H, brs)	5.94 (2H, brs)	6.18 (2H, brs)	6.71 (1H, s)
19				3.23 (3H, s)
1′			5.87 (1H, d, 7.8)	
2′			3.43 (1H, m)	
3′			3.52 (1H, m)	
4′			3.47 (1H, m)	
5′			3.39 (1H, m)	
6′			3.81 (1H, m) 4.08 (1H, m)	

those bearing an oxygen atom, while the carbons at δ_C 76.05 (s), 55.40 (t), and 48.71 (t) were assigned to those bearing a nitrogen atom. The ^{1}H–^{1}H COSY spectrum revealed connectivities of C-10–C-11, C-6–C-8, and C-1–C-4, corresponding to three partial units **a**, **b**, and **c**, respectively (Fig. 1). HMBC correlations for H_2-10 of C-8 (δ_C 55.40) and C-5 (δ_C 76.05) and H_2-8 of C-5 gave rise to the connectivity of two partial structures **a** and **b** through a nitrogen atom (N-9). In addition, HMBC correlations for H_2-1 to C-5 and C-6 (δ_C 41.84) and H-3 to C-5 suggested that the partial structure **c** was also connected through a quaternary carbon (C-5). HMBC correlations for H_2-11 to C-13 (δ_C 128.75) and C-17 (δ_C 112.08) and H-4 to C-12 (δ_C 131.48) and C-14 (δ_C 113.87) were indicative of connections among a 1,2,4,5-tetrasubstituted benzenoid ring with a methylene dioxide and units **a** and **c**. Thus, the gross structure of cephalezomine G was assigned as **1**.

Table 2. ^{13}C NMR data (δ_C) of cephalezomines G (**1**), H (**2**), J (**3**), and M (**6**) in CD_3OD at 300 K

	1	2	3	6
1	34.49	34.68	47.07	126.20
2	77.89	71.46	196.51	32.76
3	82.83	76.57	153.11	74.30
4	57.83	57.43	144.74	37.46
5	76.05	72.17	74.90	76.79
6	41.84	41.04	33.85	136.88
7	19.05	19.31	21.64	26.82
8	55.40	55.53	54.42	49.09
10	48.71	50.38	53.21	52.01
11	29.43	29.44	31.02	22.86
12	131.48	131.36	132.80	35.27
13	128.75	128.61	124.19	133.93
14	113.87	113.68	111.68	123.41
15	148.81	148.81	148.26	120.03
16	149.28	149.26	150.76	144.62
17	112.08	111.98	110.36	147.32
18	102.68	102.70	103.22	120.37
19				56.51
1′			100.05	
2′			75.09	
3′			77.83	
4′			71.38	
5′			78.73	
6′			62.54	

The NOESY spectrum of **1** showed cross-peaks as shown in computer-generated 3D drawing (Fig. 2). NOESY correlations of H-2 and H-3 to H-11a indicated that both H-2 and H-3 were β-oriented. The relative stereochemistry at

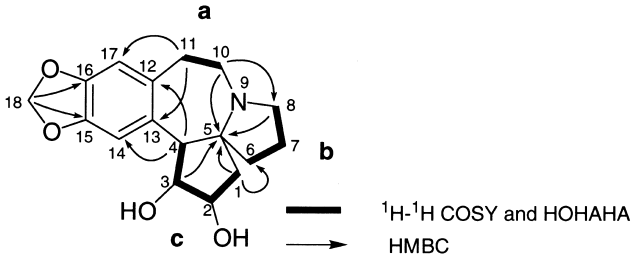

Figure 1. Selected 2D NMR correlations of cephalezomine G (**1**).

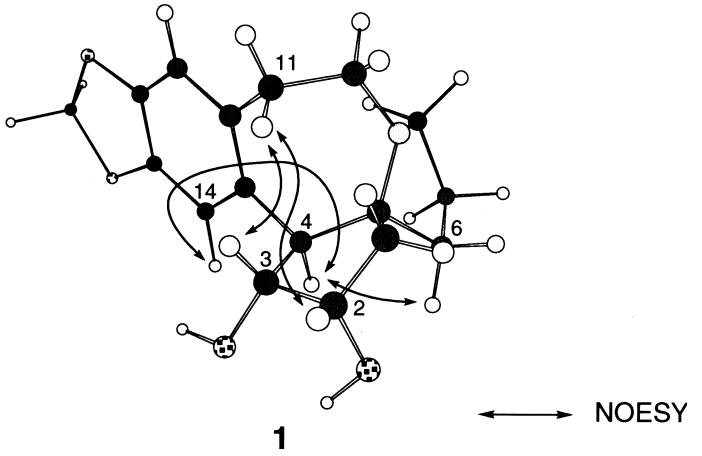

Figure 2. Selected NOESY correlations and relative sterochemistry for cephalezomine G (**1**).

C-4 and C-5 was assigned by NOESY correlations of H-4 to H-14 and H-6a. Thus, the relative stereostructure of cephaloezomine G (**1**) was assigned as shown in Fig. 2. To determine the absolute configurations at C-2 and C-3,

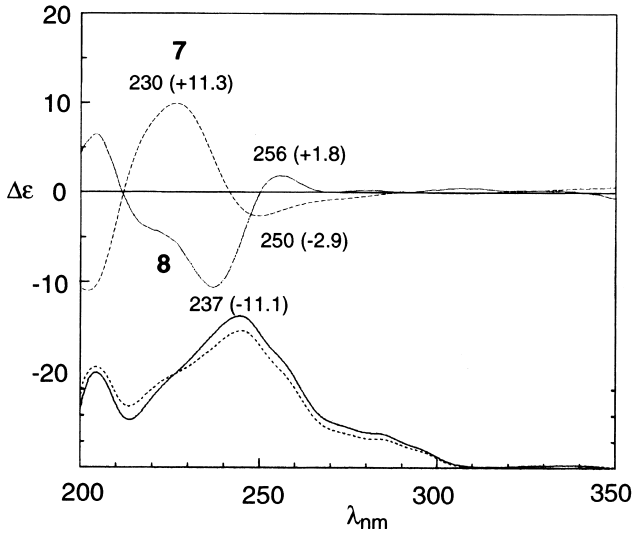

Figure 3. CD and UV spectra of binaphtyl esters (**7** and **8**).

1 was converted into its binaphthalene esters of C-2 (**7**) or C-3 (**8**) by treatment with 3-cyanocarbonyl-3′-methoxycarbonyl-2,2′-binaphthalene and an induced exciton chirality method[4] was applied for **7** and **8**. In the CD spectra, a split CD indicating a negative exciton chirality at 250 nm and a positive one at 230 nm for **7**, and that showing a positive one at 256 nm and a negative one at 237 nm for **8** were observed (Fig. 3). Conformational calculations of **7** and **8** by Monte Carlo simulation[5] suggested that in the most stable conformer, calculated screw senses between the two longitudinal electric transition moments of binaphthyl groups were in good accordance with those expected from their exciton chiralities (Fig. 4). Therefore, the absolute configurations at C-2 and C-3 were assigned as *S* and *R*, respectively.

Cephalezomine H (**2**) had the same molecular formula, $C_{17}H_{21}NO_4$, as that of **1** by HRFABMS [*m/z* 304.1537 $(M+H)^+$, $\Delta-1.2$ mmu]. The IR absorption at 3375 cm^{-1} indicated the presence of hydroxyl group(s). ^1H and ^{13}C NMR data (Tables 1 and 2, respectively) of **2** were very close to those of **1**. Detailed analysis of 2D NMR including the $^1H-^1H$ COSY, HOHAHA, HMQC and HMBC spectra indicated that **2** was a stereoisomer at C-2 and/or C-3 of **1**.

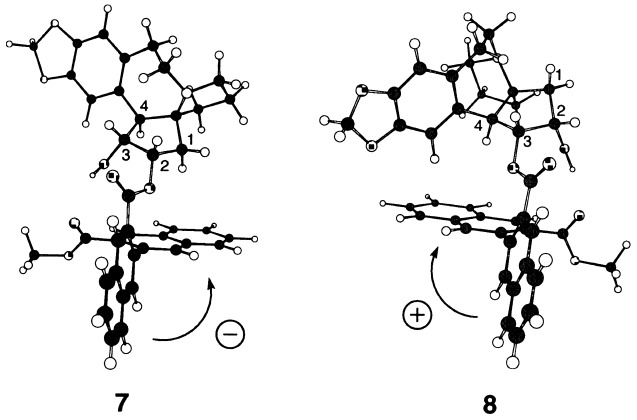

Figure 4. Each stable conformer of the binaphthyl esters (**7** and **8**) of cephalezomine G (**1**) analyzed by Monte Carlo simulation followed by minimization and clustering analysis.

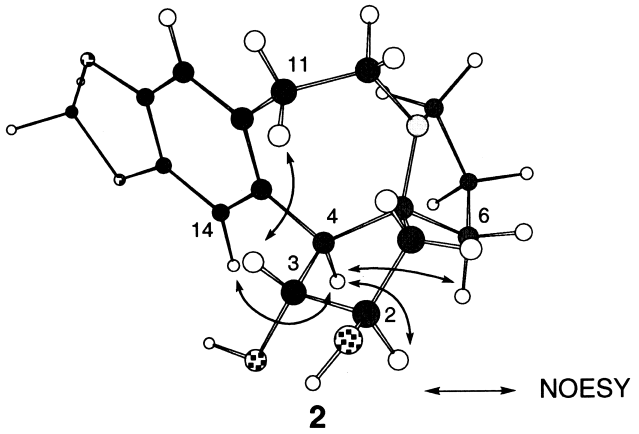

Figure 5. Selected NOESY correlations and relative stereochemistry for cephalezomine H (**2**).

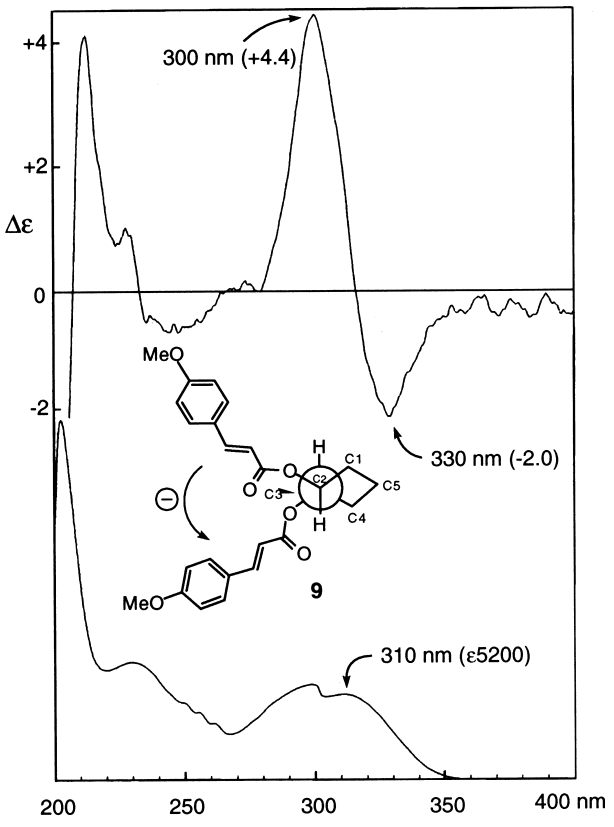

Figure 6. CD and UV spectra of 2,3-*O*-bis-*p*-methoxycinnamate (**9**) of cephalezomine H (**2**).

NOESY correlations for H-4 to H-2, H-6a, and H-14 and H-3 to H-11b in **2** indicated that the hydroxyl group at C-2 was β-oriented. The $^{1}H-^{1}H$ coupling (5.6 Hz) between H-3 and H-4 in **2** similar to that (6.6 Hz) of **1** implied that **2** possessed the same configuration at C-3 as that of **1**. The relative stereochemistry at C-4 and C-5 was assigned as the same as that of **1** by NOESY correlations as shown in Fig. 5. The absolute stereochemistry of **2** was elucidated by applying exciton chirality method[6] for **9** after introduction of *p*-methoxycinnamoyl chromophore into the hydroxyl groups at C-2 and C-3. The sign of the first Cotton effect [λ_{max} 330 nm ($\Delta\varepsilon=-2.0$)] was negative, while that of the second one [λ_{max} 300 nm (+4.4)] was positive (Fig. 6), indicating that the chirality between the two *p*-methoxycinnamoyloxy groups at C-2 and C-3 of **9** was as shown in Fig. 6 (left-handed screw). Thus, the absolute configurations at C-2 and C-3 were assigned as *R* and *R*, respectively.

Cephalezomine J {**3**, $[\alpha]_D=+80°$ (*c* 0.5, MeOH)} showed the pseudomolecular ion at *m/z* 462 $(M+H)^+$, and molecular formula, $C_{23}H_{27}NO_9$, was established by HRFABMS [*m/z* 462.1739, $(M+H)^+$, $\Delta-2.5$ mmu]. IR absorptions were attributed to hydroxyl (3360 cm^{-1}) and conjugated carbonyl (1680 cm^{-1}) groups, respectively, while the UV absorption at 235 nm implied the presence of α,β-unsaturated enone moiety. The ^{1}H and ^{13}C NMR (Tables 1 and 2, respectively) spectra of **3** were similar to those of isocephalotaxinone[7] except for carbon signals (Table 2) at δ_C 100.05, 75.09, 77.83, 71.38, 78.73, and 62.54, indicating the presence of a glucopyranose in **3**. The HMBC correlation from a proton signal at δ 5.87 to carbon signal at δ 153.11 indicated that the glucopyranose was attached to C-3. Methanolysis of **3** afforded glucose, which was elucidated to be D-glucopyranose by chiral HPLC analysis of *O*-benzoyl derivative, and demethylcephalotaxinone.[8] The glucoside linkage was assigned as β from observation of an anomeric proton signal at δ_H 5.87 (1H, d, J=7.8 Hz). Thus, the structure of cephalezomine J was elucidated to be **3**.

Table 3. ^{1}H [δ_H (J, Hz)] and ^{13}C [δ_C] NMR data of cephalezomines K (**4**) and L (**5**) in CD_3OD at 300 K

Position	δ_H		δ_C	
	4	**5**	**4**	**5**
1	5.37 s	5.36 s	95.77	95.72
2			165.38	165.36
3	6.11 d (9.6)	6.10 d (9.6)	74.29	74.27
4	4.20 d (9.6)	4.20 d (9.6)	53.08	53.05
5			78.50	78.48
6a	1.44 m	1.44 m	40.32	40.30
6b	2.25 m	2.25 m		
7a	2.00 m	1.99 m	19.84	19.83
7b	2.22 m	2.20 m		
8a	3.37 m	3.36 m	54.23	54.21
8b	3.59 m	3.58 m		
10a	3.25 m	3.24 m	48.96	48.93
10b	3.41 m	3.42 m		
11a	2.70 m	2.70 m	29.05	29.05
11b	3.33 m	3.34 m		
12			130.63	130.62
13			126.58	126.57
14	6.76 s	6.75 s	114.89	114.86
15			149.90	149.86
16			148.88	148.84
17	6.81 s	6.81 s	111.87	111.86
18	5.96 brs	5.95 brs	102.88	102.85
2-OMe	3.84 s	3.84 s	59.10	59.10
1′			174.29	174.27
2′			73.52	73.54
3′	1.96 d (16.2)	1.96 d (16.2)	44.01	43.99
	2.26 d (16.2)	2.25 d (16.2)		
4′			171.65	171.67
4′OMe	3.55 s	3.55 s	52.07	52.07
1″	1.44 m	1.44 m	40.91	40.92
	2.25 m	2.25 m		
2″	1.23 m	1.27 m	18.22	18.32
	1.47 m	1.44 m		
3″	1.38 m	1.38 m	39.46	39.47
4″			76.10	76.07
5″	3.36 brs	3.34 brs	70.39	70.25
6″	1.11 s	1.11 s	23.71	23.68

Cephalezomines K {**4**, $[\alpha]_D=-99°$ (*c* 0.6, MeOH)} and L {**5**, $[\alpha]_D=-93°$ (*c* 1.0, MeOH)} gave the same molecular formula, $C_{29}H_{39}NO_{10}$, by HRFABMS [**4**, *m/z* 562.2644, $(M+H)^+$, $\Delta-0.8$ mmu; **5**, *m/z* 562.2654, $(M+H)^+$, Δ 0.1 mmu]. IR absorptions of **4** and **5** were attributed to hydroxyl (3380 cm^{-1}) and ester carbonyl (1748 cm^{-1}) groups, respectively. The FABMS spectra of **4** and **5** showed a common fragment ion peak at *m/z* 298, characteristic for cephalotaxine-type skeleton with a side chain at C-3.[9] ^{1}H and ^{13}C NMR data (Table 3) of **4** and **5** corresponded well to those of homoharringtonine[10] except for the aliphatic ester moieties.

In the HMBC spectrum, long-range $^{1}H-^{13}C$ correlations (Fig. 7) indicated that **4** and **5** possessed a cephalotaxine-type framework. HMBC cross-peaks of H-3′ to C-1′ and C-1″, H_3–OMe (5′) to C-4′, H-5″ and H-6″ to C-3″, and

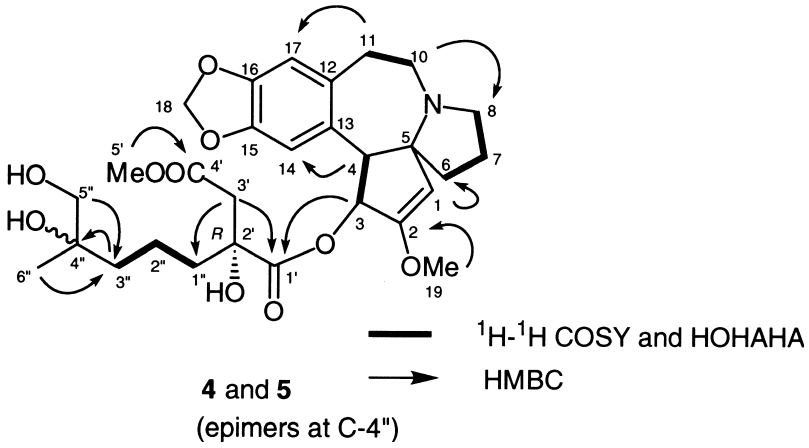

Figure 7. Selected 2D NMR correlations for cephalezomines K (**4**) and L (**5**).

H-3″ to C-4″ revealed the presence of the methyl ester of 2-(4,5-dihydroxy-4-methyl-pentyl)-2-hydroxy-succinic acid. The connectivity between C-3 and C-1′ was deduced from HMBC correlations of H-3 and H-3′ to C-1′. Thus, the structures of cephalezomines K and L were assigned as **4** and **5**, respectively, which were stereoisomers to each other.

The relative stereochemistry of **4** and **5** was deduced from NOESY data. The pentacyclic core was elucidated to have the same relative stereochemistry as that of homoharringtonine. The CD spectra for the molybdate complex with the dicarboxy acid moiety (C-1′–C-4′ and C-1″–C-6″) of the acid hydrolysates of **4** and **5** showed a negative Cotton effect at 270 nm, indicating the absolute configurations at C-2′ of **4** and **5** were *R*.[11] Thus, the stereostructures of **4** and **5**, each of which was epimer at C-4″, were elucidated as shown in the figure.

Cephalezomine M {**6**, $[\alpha]_D=+64°$ (*c* 0.8, MeOH)} showed the pseudomolecular ion at *m/z* 302 $(M+H)^+$ and the molecular formula, $C_{18}H_{23}NO_3$, was established by HRFABMS [*m/z* 302.1729, $(M+H)^+$, $\Delta-2.7$ mmu], which was smaller than that of epischellhammericine B,[12] previously isolated from the same plant,[2] by a CH_2 unit. Detailed analyses of 2D NMR spectra of **6** indicated the presence of homoerythrina-type skeleton. Treatment of **6** with trimethyldiazomethane afforded the same homoerythrina alkaloid as that obtained by methylation of epischellhammericine B.[12] Thus, the structure of cephalezomine M was elucidated to be **6**.

Cephalezomines G, H, J, K, L, and M (**1**–**6**) are new *Cephalotaxus* alkaloids having cephalotaxine- or homoerythrina-type skeletons, in which cephalezomine J (**3**) is the first example of cephalotaxine-type alkaloids with a sugar moiety. The cytotoxicity of cephalezomines G, H, J, K, L, and M (**1**–**6**) against murine lymphoma L1210 cells and human epidermoid carcinoma KB cells is shown in Table 4. In comparison of cytotoxicities among these compounds, cephalotaxine-type ones with a side chain (**4** and **5**) showed potent cytotoxicity against KB cells rather than L1210 cells, whereas those without side chain (**1** and **2**) and with a sugar moiety (**3**) exhibited relatively weak cytotoxicity.

Table 4. Cytotoxicity of cephalezomines G, H, J, K, L, and M (**1**–**6**) against murine lymphoma L1210 and human epidermoid carcinoma KB cells

Compounds	IC_{50} (μg/mL)	
	L1210	KB
1	8.0	>30
2	8.6	>30
3	12	5.6
4	1.2	0.036
5	3.6	0.044
6	>30	13

1. Experimental

1.1. General methods

^1H and 2D NMR spectra were recorded in CD_3OD on a 600 MHz spectrometer at 300 K, while ^{13}C NMR spectra were measured on a 150 MHz spectrometer. Chemical shifts were reported using residual CD_3OD (δ_H 3.31 and δ_C 49.00) as internal standards. Standard pulse sequences were employed for the 2D NMR experiments. HMBC spectra were recorded using a 50 ms delay time for long-range C–H coupling with *Z*-axis PFG. NOESY spectra were measured with a mixing time of 800 ms. FABMS was measured by using glycerol matrix.

1.2. Material

The leaves of *C. harringtonia* var. *nana* were collected in Sapporo (Hokkaido, Japan) in 2001. The botanical identification was made by Mr N. Yoshida, Health Sciences University of Hokkaido. A voucher specimen has been deposited in the herbarium of Hokkaido University.

1.3. Extraction and isolation

The leaves of *C. harringtonia* var. *nana* (13 kg) were crashed and extracted with MeOH (20 L) three times and the extract (1016 g) was treated with 3% tartaric acid to adjust pH 2 and then partitioned with AcOEt. The aqueous layer was treated with sat. Na_2CO_3 aq. to adjust pH 10 and extracted with $CHCl_3$ to give a crude alkaloidal fraction (18.3 g), in which a portion (9.1 g) was subjected to silica

gel column chromatography ($CHCl_3$/MeOH, 1:0→0:1, and then *n*-BuOH/AcOH/H_2O, 4:1:1). The fraction eluted with *n*-BuOH/AcOH/H_2O (4:1:1) was purified by C_{18} HPLC (15% CH_3CN/0.1% TFA) to afford cephalezomines G (**1**, 0.00004%) and H (**2**, 0.00002%). The fraction eluted with MeOH was subjected to C_{18} HPLC (20% CH_3CN/0.1% TFA) to give cephalezomines J (**3**, 0.00002%), K (**4**, 0.00003% yield), L (**5**, 0.00004% yield), and M (**6**, 0.00004%).

1.3.1. Cephalezomine G (1). Colorless solid; $[\alpha]_D=-48°$ (*c* 1.8, MeOH); ^1H and ^{13}C NMR (Tables 1 and 2); FABMS *m/z* 304 $(M+H)^+$; HRFABMS *m/z* 304.1539 (M+H; calcd for $C_{17}H_{22}NO_4$, 304.1549); IR (neat) ν_{max} 3395, 2920, 1490, 1225, and 1035 cm^{-1}; UV (MeOH) λ_{max} 290 (ε 2900), 239 (ε 2700), and 212 nm (ε 4900).

1.3.2. Cephalezomine H (2). Colorless solid; $[\alpha]_D=+58°$ (*c* 0.9, MeOH); ^1H and ^{13}C NMR data (Tables 1 and 2); FABMS *m/z* 304 $(M+H)^+$; HRFABMS *m/z* 304.1537 (M+H; calcd for $C_{17}H_{22}NO_4$, 304.1549); IR (neat) ν_{max} 3375, 2925, 1495, 1225, and 1040 cm^{-1}; UV (MeOH) λ_{max} 291 (ε 3100), 235 (ε 3100), and 210 nm (ε 7500).

1.3.3. Cephalezomine J (3). Colorless solid; $[\alpha]_D=+80°$ (*c* 0.5, MeOH); ^1H and ^{13}C NMR data (Tables 1 and 2); FABMS *m/z* 462 $(M+H)^+$; HRFABMS *m/z* 462.1739 (M+H; calcd for $C_{23}H_{28}NO_9$, 462.1764); IR (neat) ν_{max} 3360, 2920, 1680, 1515, 1200, and 1030 cm^{-1}; UV (MeOH) λ_{max} 312 (ε 2600), 293 (ε 2900), 250 (ε 3600), 235 (ε 4400), and 209 nm (ε 8100).

1.3.4. Cephalezomine K (4). Colorless solid; $[\alpha]_D=-99°$ (*c* 0.6, MeOH); ^1H and ^{13}C NMR data (Table 3); FABMS *m/z* 562 $(M+H)^+$; HRFABMS *m/z* 562.2644 (M+H; calcd for $C_{29}H_{40}NO_{10}$, 562.2652); IR (neat) ν_{max} 3380, 2940, 1750, 1680, and 1650 cm^{-1}; UV (MeOH) λ_{max} 289 (ε 2100), 240 (ε 2300), and 210 nm (ε 5800).

1.3.5. Cephalezomine L (5). Colorless solid; $[\alpha]_D=-93°$ (*c* 1.0, MeOH); ^1H and ^{13}C NMR data (Table 3); FABMS *m/z* 462 $(M+H)^+$; HRFABMS *m/z* 562.2654 (M+H; calcd for $C_{29}H_{40}NO_{10}$, 562.2653); IR (neat) ν_{max} 3380, 2920, 1750, 1680, and 1650 cm^{-1}; UV (MeOH) λ_{max} 289 (ε 2400), 240 (ε 2700), and 215 nm (ε 4700).

1.3.6. Cephalezomine M (6). Colorless solid; $[\alpha]_D=+64°$ (*c* 0.8, MeOH); ^1H and ^{13}C NMR data (Tables 1 and 2); FABMS *m/z* 302 $(M+H)^+$; HRFABMS *m/z* 302.1729 (M+H; calcd for $C_{18}H_{24}NO_3$, 302.1756); IR (neat) ν_{max} 3360, 2930, 1670, 1510, and 1200 cm^{-1}; UV (MeOH) λ_{max} 286 (ε 1800), 233 (ε 3600), and 217 nm (ε 4500).

1.3.7. 3′-Methoxycarbonyl-2,2′-binaphthoate (7 and 8) of cephalezomine G (1). To a solution of **1** (0.3 mg) in acetonitrile (50 μL) was added 3-cyanocarbonyl-3′-methoxycarbonyl-2,2′-binaphthalene (1.0 mg) and *N*,*N*-dimethylamino pyridine (1.0 mg). The mixture was allowed to stand at 40°C for 12 h. After evaporation of solvent, the residue was applied to C_{18} HPLC (50% CH_3CN/0.1% TFA) to give compounds **7** (0.3 mg) and **8** (0.1 mg). Compound **7**: FABMS *m/z* 642 $(M+H)^+$; HRFABMS *m/z* 642.2480 (M+H; calcd for $C_{40}H_{36}NO_7$, 642.2492); CD (MeOH) $\Delta\varepsilon_{250}=-2.9$ and $\Delta\varepsilon_{230}=+11.3$; ^1H NMR (CD_3OD) δ 3.28 (1H, d, *J*=7.4 Hz, H-1), 5.77 (1H, t, *J*=7.4 Hz, H-2), 5.37 (1H, dd, *J*=4.7, 7.4 Hz, H-3), 3.60 (1H, d, *J*=4.7 Hz, H-4), 2.17 (1H, m, H-6a), 2.31 (1H, m, H-6b), 2.44 (2H, m, H-7), 3.35 (1H, m, H-8a), 3.60 (1H, m, H-8b), 3.45 (1H, d, *J*=10.8 Hz, H-10a), 3.69 (1H, d, *J*=10.8 Hz, H-10b), 6.69 (1H, s, H-14), 7.17 (1H, s, H-17), 6.00 (1H, s, H-18a), 6.01 (1H, s, H-18b), 6.06 (1H, d, *J*=16.0 Hz), 6.10 (1H, d, *J*=16.0 Hz), 7.06 (1H, d, *J*=16.0 Hz), 7.24 (1H, d, *J*=16.0 Hz), 6.74 (2H, d, *J*=8.7 Hz), 6.86 (2H, d, *J*=8.7 Hz), 7.22 (2H, d, *J*=8.7 Hz), and 7.27 (2H, d, *J*=8.7 Hz). Compound **8**: FABMS *m/z* 642 $(M+H)^+$; HRFABMS *m/z* 642.2471 (M+H; calcd for $C_{40}H_{36}NO_7$, 642.2492); CD (MeOH) $\Delta\varepsilon_{256}=+1.8$ and $\Delta\varepsilon_{237}=-11.1$; ^1H NMR (CD_3OD) δ 3.28 (1H, d, *J*=7.4 Hz, H-1), 5.77 (1H, t, *J*=7.4 Hz, H-2), 5.37 (1H, dd, *J*=4.7, 7.4 Hz, H-3), 3.60 (1H, d, *J*=4.7 Hz, H-4), 2.17 (1H, m, H-6a), 2.31 (1H, m, H-6b), 2.44 (2H, m, H-7), 3.35 (1H, m, H-8a), 3.60 (1H, m, H-8b), 3.45 (1H, d, *J*=10.8 Hz, H-10a), 3.69 (1H, d, *J*=10.8 Hz, H-10b), 6.69 (1H, s, H-14), 7.17 (1H, s, H-17), 6.00 (1H, s, H-18a), 6.01 (1H, s, H-18b), 6.06 (1H, d, *J*=16.0 Hz), 6.10 (1H, d, *J*=16.0 Hz), 7.06 (1H, d, *J*=16.0 Hz), 7.24 (1H, d, *J*=16.0 Hz), 6.74 (2H, d, *J*=8.7 Hz), 6.86 (2H, d, *J*=8.7 Hz), 7.22 (2H, d, *J*=8.7 Hz), and 7.27 (2H, d, *J*=8.7 Hz).

1.3.8. 2,3-*O*-Bis-*p*-methoxycinnamate (9) of cephalezomine H (2). To a solution of **2** (0.1 mg) in pyridine (100 μL) was added *p*-methoxycinnamoyl chloride (1.0 mg) and *N*,*N*-dimethylamino pyridine (0.3 mg). The mixture was allowed to stand at 50°C for 20 h. The residue was dissolved in $CHCl_3$ and washed with H_2O. After evaporation of solvent, the residue was applied to C_{18} HPLC (50% CH_3CN/0.1% TFA) to give a compound (**9**, 0.1 mg); FABMS *m/z* 624 $(M+H)^+$; HRFABMS *m/z* 624.2611 (M+H; calcd for $C_{37}H_{38}NO_8$, 624.2597); IR (neat) ν_{max} 3350, 2925, 1710, 1180, and 1030 cm^{-1}; UV (MeOH) λ_{max} 310 (ε 5200), 298 (ε 5800), and 229 nm (ε 7100); CD (MeOH) $\Delta\varepsilon_{329}=-2.0$ and $\Delta\varepsilon_{300}=+4.4$; ^1H NMR (CD_3OD) δ 3.82 (3H, s), 3.84 (3H, s), 5.89 (2H, m), 5.96 (2H, m), 6.09 (1H, d, *J*=15.7 Hz), 6.22 (1H, d, *J*=15.7 Hz), 6.66 (2H, brs), 6.84 (2H, d, *J*=8.5 Hz), 6.89 (2H, d, *J*=8.5 Hz), 7.39 (4H, m), 7.47 (1H, d, *J*=15.7 Hz), 7.62 (1H, d, *J*=15.7 Hz).

1.4. Determination of stereochemistry of the sugar unit in cephalezomine J (3) by chiral HPLC

Cephalezomine J (**3**, 0.1 mg) was treated with 3% HCl/MeOH (200 μL) at 90°C for 1 h. After the solvent was removed by nitrogen stream, to the residue was added $CHCl_3$ (100 μL), and the $CHCl_3$ solution was extracted with H_2O (100 μL×3). The aqueous fraction evaporated in vacuo was treated pyridine (100 μL), triethylamine (10 μL), and benzoyl chloride (10 μL), at room temperature for 20 h. After addition of MeOH (100 μL), the reaction mixture was extracted with hexane (100 μL×3). The hexane-soluble fraction was evaporated in vacuo to afford *O*-benzoyl/methyl derivative of the sugar units of **3**. Authentic D- and L-glucose were treated with benzoyl chloride as described above to afford *O*-benzoyl/methyl derivatives of D- and L-glucose, respectively. The *O*-benzoyl/methyl derivatives were subjected to chiral

HPLC analyses using Chiralpak OP (+) (Daicel Chemical Industry, Ltd., 4.6×250 mm). The retention time of *O*-benzoyl/methyl derivatives of methanolysis product of **3** was found to be 16.2 min, while the retention times of *O*-benzoyl/methyl derivatives of authentic D- and L-glucose were found to be 16.2 and 17.2 min, respectively.

1.5. Molybdate complexes of hydrolysates of cephalezomines K and L (4 and 5)

Each of compounds **4** and **5** (1 mg) was hydrolyzed with 3N HCl (1 mL) under reflux for 4 days. After cooling, 3 M NH_4OH was added and the alkaline phase was extracted with $CHCl_3$. Excess NH_4OH was neutralized and the solvent was evaporated under reduced pressure. The residue was used directly in the preparation of solution for CD measurement, which contained 3 mM each hydrolysates of **4** and **5** and 2.7 mM Na molybdate. HCl and NaOH solution were added until pH 2.9–3.1 was reached. Measurements of CD spectra were carried out in a 1 mm cell 5 days after the solution had been prepared.

1.6. Computational methods

Conformational searching was carried out using Pseudo Monte Carlo simulation in Macromodel program for **7**. One thousand Monte Carlo steps were performed, yielding 245 unique conformations in the energy region of 0–50 kJ/mol, which could be classified into two clusters. Each conformer was finally minimized by molecular mechanics calculation of MMFF force field.[13] Conformational searching for **8** was performed by the same procedure as **1** and classified into two clusters. The lowest energy conformation in each cluster was shown in Fig. 4.

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